

Pathogenic Yield of Genetic Testing in Autism Spectrum Disorder

Holly K. Harris, MD,^{a,b} Georgios D. Sideridis, PhD,^a William J. Barbaresi, MD,^a Elizabeth Harstad, MD, MPH^a

abstract

BACKGROUND AND OBJECTIVES: Genetic testing is recommended for individuals with autism spectrum disorder (ASD). Pathogenic yield varies by clinician and/or patient characteristics. Our objectives were to determine the pathogenic yield of genetic testing, the variability in rate of pathogenic results based on subject characteristics, and the percentage of pathogenic findings resulting in further medical recommendations in toddlers with a *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* diagnosis of ASD.

METHODS: We conducted a retrospective chart review of 500 toddlers, 18 to 36 months, diagnosed with *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* ASD (mean age: 25.8 months, 79% male). Subject demographics, medical and neuropsychological characteristics, and genetic test results were abstracted. Genetic results were divided into negative or normal, variants of unknown significance, and pathogenic. Subject characteristics were compared across results. Manual chart review determined if further recommendations were made after pathogenic results.

RESULTS: Over half of subjects (59.8%, $n = 299$) completed genetic testing, and of those, 36 (12.0%) had pathogenic findings. There were no significant differences in Bayley Scales of Infant Development cognitive ($P = .112$), language ($P = .898$), or motor scores ($P = .488$) among children with negative or normal findings versus a variant of unknown significance versus pathogenic findings. Medical recommendations in response to the genetic finding were made for 72.2% of those with pathogenic results.

CONCLUSIONS: Our findings reinforce the importance of genetic testing for toddlers diagnosed with ASD given the 12% yield and lack of phenotypic differences between subjects with and without pathogenic findings. The majority of pathogenic results lead to further medical recommendations.



^aDivision of Developmental Medicine, Boston Children's Hospital and Harvard Medical School, Harvard University, Boston, Massachusetts; and ^bDepartment of Pediatrics, Baylor College of Medicine and Meyer Center for Developmental Pediatrics, Texas Children's Hospital, Houston, Texas

Drs Harris, Barbaresi and Harstad conceptualized and designed the study; Dr Harris designed the data collection instruments, collected data, and drafted the initial manuscript; Dr Sideridis conducted the statistical analyses and reviewed and revised the manuscript; Drs Harris, Sideridis, Barbaresi, and Harstad reviewed and revised the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

DOI: <https://doi.org/10.1542/peds.2019-3211>

Accepted for publication Jul 28, 2020

Address correspondence to Elizabeth Harstad, MD, MPH, Division of Developmental Medicine, Boston Children's Hospital, Fegan 10, 300 Longwood Ave, Boston, MA 02115. E-mail: elizabeth.harstad@childrens.harvard.edu

WHAT'S KNOWN ON THIS SUBJECT: Genetic testing (chromosomal microarray and fragile X) is recommended for patients with autism spectrum disorder (ASD). Reported pathogenic yield is 10% for chromosomal microarray and 1 to 5% for fragile X. The pathogenic yield in toddlers diagnosed with ASD is unknown.

WHAT THIS STUDY ADDS: In a clinical sample of 500 toddlers with *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* ASD, 299 (59.8%) completed genetic testing, and of those, 36 (12.0%) had pathogenic findings. Pathogenic findings impacted medical decision-making 72.2% of the time.

To cite: Harris HK, Sideridis GD, Barbaresi WJ, et al. Pathogenic Yield of Genetic Testing in Autism Spectrum Disorder. *Pediatrics*. 2020;146(4):e20193211

Autism spectrum disorder (ASD) is marked by deficits in social communication and the presence of restricted and repetitive behaviors.¹ ASD is heritable, with a high rate of concordance of diagnoses in monozygotic twins and increased rates among siblings.^{2–4} Certain genetic syndromes have an increased rate of co-occurring ASD, including fragile X, tuberous sclerosis, and Rett syndrome, among others. However, these known disorders account for a small proportion of overall ASD cases.^{5–8} The American College of Medical Genetics guideline recommends chromosomal microarray (CMA) for all patients with ASD and fragile X testing in boys.⁹ These recommendations were based on reported pathogenic yield for these tests, ~10% for CMA and 1% to 5% for fragile X.⁹ Although the percentages are not high, the yield is relatively high for a behaviorally diagnosed disorder without a clear biomarker or diagnostic test. In the American Academy of Pediatrics 2019 ASD clinical guidelines, genetic testing is recommended for all children with ASD.

Variation in pathogenic yield is dependent on several factors, including age, with toddlers demonstrating lower yield compared with other age groups.^{10–14} Patients with the narrow, more-severe presentation of autistic disorder have higher yield compared with broader ASD phenotypes within the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.^{11,13} Whether yield has changed after changes in the ASD diagnostic criteria remains to be determined. Previous work suggests that yield, particularly for pathogenic de novo mutations, is higher in patients with “syndromic” or “complex” ASD, describing individuals with ASD and features such as intellectual disability, dysmorphism, or congenital anomalies.^{4,5,12,14–16} However, researchers of some studies found no

significant differences when comparing medical and neuropsychological features between patients with versus without pathogenic findings.^{4,5,12,14–17}

Despite clinical recommendations to complete genetic testing in individuals with ASD, insurance coverage varies. Although public insurers often reimburse fully for testing, private insurer reimbursement is variable, with testing completely covered, not covered, or covered in a limited manner, resulting in out-of-pocket expense for families. Variation in insurance coverage may occur because little is known about how often a pathogenic finding results in further clinical recommendations. Determining the rate at which genetic findings result in further medical recommendations would improve clinician and patient understanding of potential outcomes after pathogenic results. Should further recommendations be frequent, this would support the current guidelines for testing, inform clinical care, and encourage third-party payer support for coverage of genetic testing in this population. To our knowledge, there are no studies that report the rate at which further medical recommendations are made on the basis of pathogenic genetic results. Our objectives in this study are to understand differences between those who do and do not complete genetic testing, determine the pathogenic yield of genetic testing in a clinical sample of toddlers with a *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) diagnosis of ASD, compare medical and neuropsychological characteristics among patients with differing genetic findings (normal or no pathogenic finding versus variant of unknown significance versus pathogenic), and determine the rate at which pathogenic findings result in subsequent medical recommendations.

METHODS

Sample

We conducted a retrospective chart review of 500 toddlers (18–36 months) with an ASD diagnosis made in the Developmental Medicine Center at Boston Children’s Hospital (BCH) via an interdisciplinary team assessment from July 1, 2013, to May 1, 2016. Five children were excluded because they were seen by a single clinician, rather than a team, for the initial ASD evaluation. In the team assessments, a developmental-behavioral pediatrician obtained a medical and developmental history and conducted a physical examination and a clinical child psychologist obtained neuropsychological measures. Diagnoses were formulated and agreed on by the pediatrician and psychologist through a clinical team meeting that occurred immediately after the diagnostic assessment and through which consensus diagnoses were made. The Bayley Scales of Infant and Toddler Development, Third Edition (Bayley)¹⁸ was used to obtain cognitive, language, and motor standard scores. The Vineland Adaptive Behavior Scales, Second or Third Editions^{19,20} were used to obtain scores related to adaptive functioning. The time frame for data abstraction corresponded with a clinic-wide shift to use of a checklist to ensure fidelity to the new DSM-5 criteria.²¹ We identified subjects via electronic medical record search based on a billing code signifying a team assessment (99245 AND 96118 or 96119) and an *International Classification of Diseases, Ninth Revision* or *10th Revision* code for ASD (F84.0, F84.9, or F84.5 or 299.0, 299.8, or 299.9). Inclusion criteria were a documented DSM-5 diagnosis of ASD made via a team assessment. Patients are referred to the center by primary care clinicians, early childhood intervention providers, or parents on the basis of concern for delayed or atypical development. It is standard

practice for clinicians in this practice to recommend genetic testing (CMA and fragile X) for all children diagnosed with ASD. The study was approved by the BCH Institutional Review Board with a waiver of informed consent.

Data Collection Procedures

We abstracted demographic, medical, and neuropsychological information for all subjects (Table 1). We recorded whether subjects completed genetic testing, along with the results of all CMA and fragile X testing. CMA analysis was performed via custom whole genome array with enhanced probe coverage on targeted genes and with single nucleotide polymorphism or loss of heterozygosity probes (Optimized Version 3.0 on Agilent 180k platform). The array uses human genome build 19, has a resolution of ~50 kb for targeted regions or genes, and can detect copy number changes as small as ~150 kb between targeted regions. CMA findings were reported as follows: (1) no clinically significant copy number variants or areas of copy number neutral homozygosity (≥ 10 Mb) were

identified (which we refer to as “negative or normal”), (2) variants of unknown or uncertain significance, and (3) known or likely pathogenic finding. For fragile X testing, DNA was amplified across the region of CGG repeats in Fragile X mental retardation 1 by using Asuragen Amplidex Fragile X mental retardation 1 polymerase chain reaction reagents. Fragile X results were reported by CGG repeats, categorized as (1) normal (5–44 repeats), (2) “grey zone” (45–54 repeats), (3) premutation (55–200 repeats), and (4) full mutation (>200 repeats). All tests were performed at Claritas Genomics and followed the requirements of Clinical Laboratory Improvement Amendment 88 for clinical testing. Analyses were performed on DNA extracted by standard methodologies from blood samples. Results were reviewed, and the pathogenicity of those results determined by laboratory geneticists with Claritas Genomics. Pathogenicity determination was included in the report that was returned to the ordering clinician who, in turn, communicated the results to the

parent. For pathogenic findings, clinicians often referred to a geneticist who then made additional, specific recommendations. For purposes of analyses, we considered known or likely pathogenic CMA findings and any abnormal fragile X findings to be “pathogenic.”

For patients with a pathogenic CMA finding, we performed further manual chart review to determine if a medical recommendation was subsequently made on the basis of the genetic result. The following documented medical recommendations would not have otherwise been made before the return of a pathogenic result: referral to other specialists (endocrinology, metabolism, and sleep medicine in our sample), audiology examination, ophthalmology examination, monitoring for seizures, echocardiogram, renal ultrasound, or other laboratory evaluation (urine amino acids, creatine kinase level). For pathogenic CMA results, referral to genetics, parental testing, or genetic counseling were not considered a recommendation. Those with a pathogenic finding on fragile X testing were referred to a fragile X clinic. This was considered a further medical recommendation because this referral informed family planning and resulted in ongoing monitoring. Although genetic testing is recommended at the time of diagnosis, factors such as family preference, logistics, and insurance authorization often delay testing. Data abstraction occurred at least 2 to 3 years after the diagnosis of ASD and thus reflects any genetic testing that occurred following the ASD diagnosis. Two trained reviewers (including author H.H.) recorded data in the Research Electronic Data Capture (REDCap)²² database with $>96\%$ interrater reliability as determined by bimonthly review of 10% of the records that were double-coded. A third reviewer (E.H.) arbitrated any coding discrepancies.

TABLE 1 Variables Abstracted From Medical Record

Demographic Characteristics	Medical Characteristics	Neuropsychological Characteristics
Age at diagnosis	Gestational Age	Verbal ^b or nonverbal
Sex	Regression ^c	Bayley cognitive standard score
Insurance	Epilepsy	Bayley language standard score
Mean household income ^a	Head Circumference	Bayley motor standard score
	Family history of ASD	Vineland adaptive behavior composite
	Dysmorphic features reported by clinician	Vineland communication standard score
	Hypotonia	Vineland motor standard score
	Additional diagnosis of global developmental delay (Bayley cognitive standard score <70)	Vineland socialization standard score

To evaluate the potential biased effects of missing data, a series of tests evaluated whether missing data participants (on the dependent variables) were disproportionately allocated to the two groups of interest (genetic testing or not). Results found by using a series of χ^2 tests pointed to the adoption of null hypotheses only. Consequently, the amount of missing data were approximately equally distributed across individuals with genetic testing scores and without. The interested reader may consult Supplemental Information.

^a Estimated via census tracts based on subject zip code at diagnosis.
^b At least 1 consistent, recognizable word.
^c Clinician concern for regression which was indicated by referral to a subspecialist (neurology or metabolism and/or genetics) or further metabolic laboratory studies.

TABLE 2 Comparison Between Subjects Who Completed Genetic Testing and Patients Not Completing Genetic Testing Across Demographic and Medical Characteristics

	Patients Completing Genetic Testing (<i>n</i> = 299)	Patients Not Completing Genetic Testing (<i>n</i> = 201)	Statistic ^a	<i>P</i> ^a	Effect Size ^b
Demographic characteristics					
Age (mean, SD)	25.84 (4.4)	26.21 (4.5)	0.92	.36	−0.08 (−0.26 to 0.10)
Female, <i>n</i> (%)	65 (21.7)	39 (19.4)	0.40	.53	0.12
Insurance, <i>n</i> (%)			3.03	.22	0.16
Private	164 (54.9)	126 (62.7)			
Public	126 (42.1)	70 (34.8)			
Other	9 (3.0)	5 (2.5)			
Mean income	85 077.82	82 889.39	0.69	.49	0.06
Medical characteristics, <i>n</i> (%)					
Premature (GA ≤36)	64 (21.4)	48 (23.9)	0.42	.52	0.12
Regression	14 (4.7)	6 (3.0)	0.90	.34	0.43
Epilepsy	3 (1.0)	3 (1.5)	0.24	.62	0.41
Family history of ASD	89 (29.8)	57 (28.4)	0.12	.73	
Dysmorphic features reported by clinician	21 (7.0)	6 (3.0)	3.84 ^c	.05 ^{c,d}	0.81
Additional diagnosis of global developmental delay	125 (41.8)	74 (36.8)	0.21	.65	0.07
Neuropsychological characteristics					
Verbal, <i>n</i> (%)	160 (53.5)	128 (63.7)	5.09 ^c	.02 ^c	0.27
Bayley cognitive standard score, mean (SD), <i>n</i>	79.77 (14.16), 294	81.81 (14.83), 193	1.53	.13	−0.14
Bayley language standard score, mean (SD), <i>n</i>	61.45 (12.51), 213	65.35 (16.69), 132	2.31 ^c	.02 ^c	−0.23
Bayley motor standard score, mean (SD), <i>n</i>	78.60 (13.86), 212	79.36 (12.10), 135	0.52	.60	−0.06
Vineland adaptive behavior composite, mean (SD), <i>n</i>	73.35 (8.38), 252	76.55 (9.90), 175	3.61 ^c	.001 ^c	−0.32
Vineland communication standard score, mean (SD), <i>n</i>	69.54 (11.89), 264	73.85 (13.61), 180	3.45 ^c	.001 ^c	−0.32
Vineland motor standard score, mean (SD), <i>n</i>	85.57 (10.78), 261	87.62 (9.77), 178	2.04 ^c	.04 ^c	−0.21
Vineland socialization standard score, mean (SD), <i>n</i>	73.28 (7.27), 265	76.62 (9.72), 179	3.92 ^c	.001 ^c	−0.34

^a *P* values are for independent sample *t* tests for continuous dependent variables or χ^2 tests whenever both variables were categorical.

^b Effect size indicators are Glass δ statistic, which is a variant of Cohen *d*; estimates of 0.2, 0.5, and 0.8, respectively, are indicative of small, medium, and large effects. Estimates for the χ^2 tests were also transformed on the SD metric, and consequently, Cohen rules apply to them as well.

^c Signifies statistically significant finding with *P* < .05 when it appears in the absence of footnote “d.”

^d Finding does not exceed levels of significance using the Benjamini–Hochberg correction using a false discovery rate of 0.20.

Statistical Analyses

We calculated frequencies and percentages to represent the baseline characteristics of the sample. We used independent sample *t* tests to evaluate for significant differences between patients who completed versus did not complete genetic testing on continuous variables and

χ^2 tests for categorical variables. Power levels were in excess of 99% for either test (Supplemental Information). Effect sizes involved Glass δ statistic, which is a variant of Cohen *d*, for which values of 0.2, 0.5, and 0.8 are indicative of small, medium, and large effects, respectively. In hypotheses involving

percentages, χ^2 statistics were also presented with this effect size metric by using SDs. Thus, effects are comparable across all tests.

RESULTS

Among subjects in the study (*N* = 500, 79.2% male), 299 (59.8%) completed genetic testing (mean age: 25.8 months at diagnosis). There were no differences in demographic characteristics between those children that completed genetic testing versus those that did not (Table 2). Subjects who completed testing were more likely to have dysmorphic features, be nonverbal, and have lower Bayley language scores and lower scores on parent report of overall adaptive functioning, communication, motor, and socialization skills (Table 2).

Of the 299 subjects who completed testing, 186 (62.2%) had negative or normal findings, 77 (25.8%) had variants of unknown significance on CMA, and 36 (12%) had a pathogenic finding (Fig 1). Of those with pathogenic findings, 27 subjects had pathogenic variants on CMA (9.0%) and 9 subjects had pathogenic fragile X findings (3.0%). The most common copy number variants in this sample were deletions or duplications on 15q (*n* = 10) and 22q (*n* = 2). Among subjects with fragile X findings, there were 3 full mutations, 3 premutations, 2 intermediate or “grey zone” mutations, and 1 patient with mosaicism (Table 3). The role of fragile X premutations, “grey zone” mutations, and mosaicism in ASD is an ongoing area of investigation. Because current literature suggests potential relevance, we considered these mutations pathogenic.^{23–25}

There were no significant differences in cognitive, language, or motor standard scores or in patient age, insurance, or median household income across genetic result categories (Table 4). Among subjects

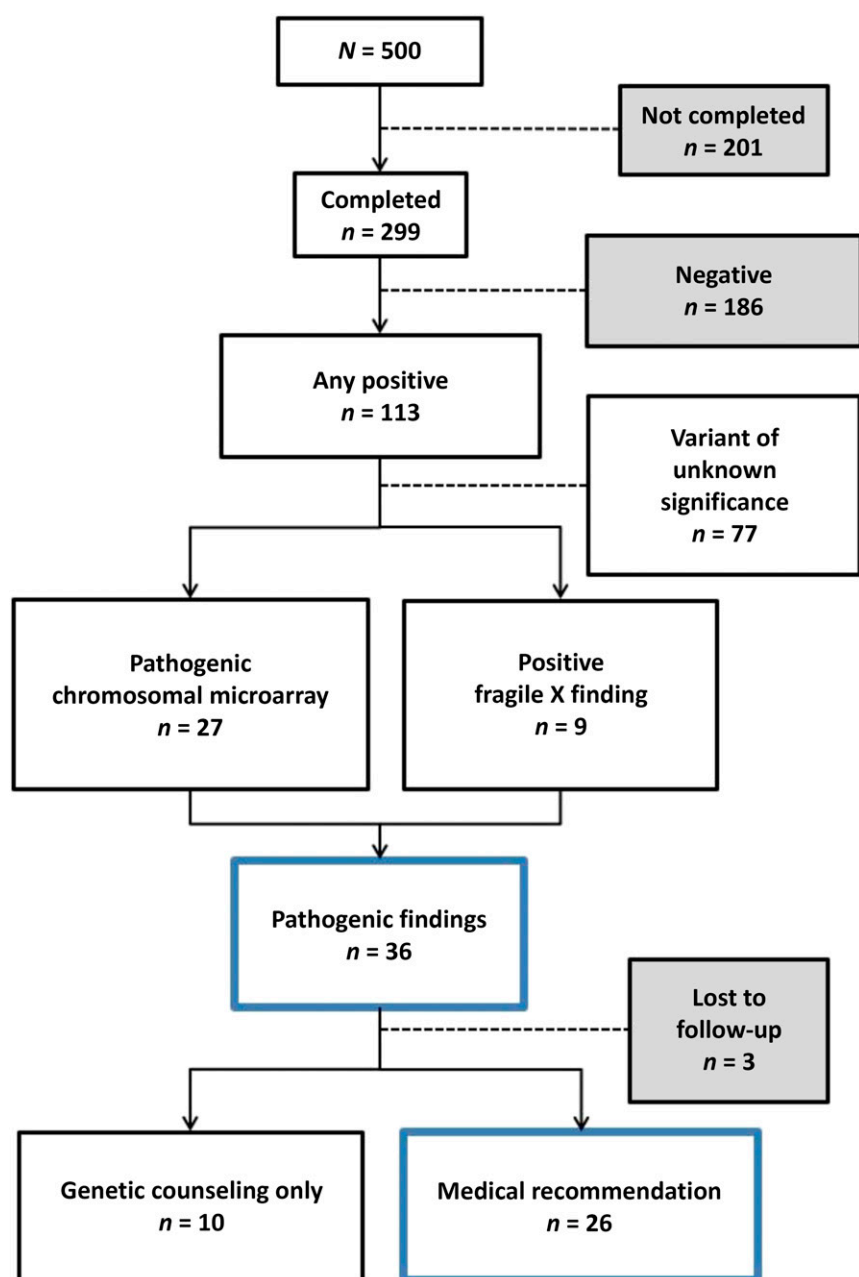


FIGURE 1
Flowchart breakdown of genetic testing completion, results, and subsequent action.

who completed genetic testing, there was a higher rate of variants of unknown significance for girls versus boys (25 of the 65 girls, 38.5%; compared with 52 of the 234 boys, 22.2%; $P = .03$). There was a significant difference in mean motor standard score on the Vineland Adaptive Behavior Scales across genetic finding categories (86.54 for negative or normal finding, 85.60 for

variants of unknown significance, and 80.10 for pathogenic findings; $P = .01$).

Of those children with a pathogenic finding ($n = 36$), manual chart review was conducted to determine if a medical recommendation was made on the basis of the finding. All children with positive fragile X results ($n = 9$) received a further

recommendation via referral to a fragile X clinic for genetic counseling and comprehensive care. Of subjects with pathogenic CMA findings ($n = 27$), 17 children (63%) had a medical recommendation made on the basis of their finding(s). The most common medical recommendations included education regarding risk and management of potential seizures and seizure monitoring ($n = 5$) and referrals to subspecialists (endocrinology, metabolism, or sleep medicine; $n = 4$). Other common recommendations included echocardiogram, renal ultrasound, audiology examination, and ophthalmology examination ($n = 3$ for each). In summary, including both pathogenic CMA findings and positive fragile X findings, 26 of 36 subjects (72.2%) had subsequent medical recommendations made on the basis of their results.

DISCUSSION

In this study, 59.8% of toddlers with a DSM-5 diagnosis of ASD completed genetic testing. Many factors may influence completion rate, including clinician practices or family considerations such as the likelihood of a positive result, the potential for results with unclear significance, or family planning implications. Additional barriers include discomfort of phlebotomy, cost, and third-party payer policies. Although the rate of completed testing in our sample is higher than previously reported (parent surveys report rates of 28%–41%), it is low in the context of guidelines that recommend genetic testing in all patients with a new ASD diagnosis.^{26,27} In this sample, parents' likelihood to pursue genetic testing for their child did not vary on the basis of subject age, insurance status, sex, or median household income. Patients who had dysmorphic features, were nonverbal, and had lower language scores were more likely to complete testing. Additionally, for those children

TABLE 3 Pathogenic Findings

Genetic Findings in Sample According to Test Performed	<i>n</i> (%)
CMA completed	299
Variants of unknown significance	77 (25.8)
Known pathogenic or likely pathogenic finding	27 (9.0)
15q deletions or duplications	10
22q11 deletions or duplications	2
The remaining were found in a single patient:	1
48 XYY	
Mosaic trisomy 8	
1p12 deletion	
2p21 deletion	
2p22.3-22.2 deletion	
2q12.3-q13 deletion	
2q37.3 deletion	
4q21.21 deletion	
5p15.33-p15.31 deletion	
5q11.2-13.1 deletion	
8p22 deletion	
13q32.3 deletion	
16p13.11 deletion	
20p11.23-p11.21 deletion	
Xp21.1 duplication	
Fragile X test completed	299
Positive finding	9 (3.0)
Full mutation	3
Premutation	3
Gray zone	2
Mosaic	1

completing testing, parent report across domains of functioning (adaptive, communication, motor, social) was lower compared with children who did not complete testing.

Among subjects who did complete CMA and fragile X testing, the pathogenic yield of 12.0% is consistent with previous reports.^{9,14,28} We did not find significant differences in age at diagnosis, insurance status, or median household income between those with and without pathogenic findings. There was a higher percentage of female subjects in the group with variants of unknown significance. This finding may be consistent with the hypothesis that girls require a higher burden of mutations to reach a threshold required to manifest ASD, known as the female protective effect.²⁹ Ho et al¹⁵ also demonstrated

a higher rate of pathogenic mutations in female patients with complex ASD.

There were no differences in medical characteristics of interest, nor was there a statistically significant difference in Bayley cognitive scores across subjects in the 3 categories of genetic test results (normal or negative findings, variants of unknown significance, and pathogenic findings). This finding is in contrast to previous work suggesting a higher pathogenic yield of testing in complex ASD, which often includes intellectual disability or cognitive delay, but is consistent with a recent study by McGrew et al,¹⁷ who compared characteristics between children with and without pathogenic findings and found no differences.^{10,12,14} Although measuring cognitive functioning in young children is challenging, our results suggest that clinicians should continue to recommend genetic testing regardless of cognitive level.

In our sample, children with known pathogenic findings had lower parent-reported motor functioning than children with negative or normal results or variants of unknown significance. This raises the question of the role of motor delays in suggesting a potential underlying genetic etiology, an area of recent investigation.³⁰ However, although this difference was statistically significant in our sample, scores in both groups remained in the “low adequate” to “moderately low” range on the Vineland Scales; such differences may not be appreciable clinically.

In children with pathogenic CMA or fragile X findings, 72.2% had further medical recommendations made on the basis of their genetic results. We combined fragile X and CMA when reporting on percentage receiving medical recommendations given our focus on implications of genetic testing in ASD overall. All subjects with abnormal fragile X results

received referral to a genetic specialist. For CMA alone, 17 of 27 (63%) received medical recommendations. Additionally, all patients with a pathogenic finding received genetic counseling around family planning. When considering the entire group of subjects (*N* = 299) who completed genetic testing, 8.7% had a medical recommendation made on the basis of their results. This finding is consistent with one previous report of 6.2%.¹⁴ Our results suggest that genetic test findings do not merely provide information for families but also result in further medical recommendations and monitoring, arguing for broader, more uniform third-party payer coverage. Furthermore, a pathogenic genetic finding may provide an answer for families seeking to understand why their child has ASD.

A potential limitation to this study is our retrospective methodology; thus, data are limited to information documented in the medical record. For example, some demographic information (race, ethnicity, maternal education) was not routinely collected. All genetic testing available for review was performed at the BCH laboratory. It is possible that some children may have had genetic testing conducted at other approved laboratories and that these results were not documented in their medical records. Although all clinicians within the practice are instructed to recommend testing and documented such recommendations in the note, the strength with which the recommendation was communicated verbally cannot be confirmed. Only 60% of subjects completed genetic testing, and on the basis of our comparisons, it is possible that the perception of greater impairment on the part of parent and/or clinician increased the likelihood that a patient completed testing, which could have influenced our findings; however, differences between those who did versus did not

TABLE 4 Comparison Between Subjects With Pathogenic Findings, Variants of Unknown Significance, and Normal Results

	G1: Negative or Normal Results (<i>n</i> = 186)	G2: Variant of Unknown Significance (<i>n</i> = 77)	G3: Known Pathogenic Finding (<i>n</i> = 36)	Statistic ^a	<i>P</i> ^a	Effect Size ^b G1 Versus G2	Effect Size ^b G1 Versus G3	Effect Size ^b G2 Versus G3
Demographic characteristics								
Age, mean (SD)	25.78 (4.5)	26.35 (4.1)	25.03 (4.5)	1.13	.33	−0.13	0.17	0.29
Female (<i>n</i> = 65, 21.7%), <i>n</i> (%)	32 (49.2)	25 (38.5)	8 (12.3)	7.15 ^c	.03 ^c	0.32	0.32	0.32
Male (<i>n</i> = 234, 78.3%), <i>n</i> (%)	154 (65.8)	52 (22.2)	28 (12.0)	—	.03 ^c	0.32	0.32	0.32
Insurance								
Private, <i>n</i> (%)	101 (43.3)	40 (51.3)	23 (65.7)	—	—	—	—	—
Public, <i>n</i> (%)	81 (43.5)	33 (42.3)	12 (34.3)	—	—	—	—	—
Other, <i>n</i> (%)	4 (2.2)	5 (6.4)	0 (0.0)	—	—	—	—	—
Mean income	84 630.11	92 605.44	88 192.85	1.65	.19	−0.23	−0.11	0.14
Medical characteristics, <i>n</i> (%)								
Premature (GA ≤36)	44 (23.7)	15 (19.2)	5 (14.3)	1.83	.40	0.34	0.34	0.34
Regression	9 (4.8)	2 (2.6)	3 (8.6)	1.98	.37	0.78	0.78	0.78
Epilepsy	3 (1.6)	0 (0.0)	0 (0.0)	1.84	.40	2.52	2.52	2.52
Family history of ASD	53 (28.5)	29 (37.2)	7 (20.0)	3.79	.15	0.45	0.45	0.45
Sibling with ASD	17 (9.1)	5 (6.4)	5 (14.3)	3.83	.43	0.81	0.81	0.81
Dysmorphic features reported by clinician	9 (4.8)	7 (9.0)	5 (14.3)	4.64	.10	1.07	1.07	1.07
Diagnosis of global developmental delay	79 (42.5)	28 (35.9)	18 (51.4)	4.05	.40	0.37	0.37	0.37
Diagnosis of hypotonia	8 (4.3)	4 (5.1)	1 (2.9)	4.27	.37	1.40	1.40	1.40
Neuropsychological characteristics								
Verbal, <i>n</i> (%)	99 (53.2)	47 (60.3)	14 (40.0)	4.00	.14	0.32	0.32	0.32
Bayley cognitive standard score, mean (SD), <i>n</i>	80.47 (14.34), 184	80.20 (13.75), 76	75.00 (13.60), 34	2.21	.11	0.02	0.40	0.38
Bayley language standard score, mean (SD), <i>n</i>	61.37 (13.05), 135	62.04 (11.70), 52	60.69 (11.53), 26	0.11	.90	−0.06	0.06	0.12
Bayley motor standard score, mean (SD), <i>n</i>	79.24 (14.18), 133	78.47 (13.05), 51	75.79 (13.90), 28	0.72	.49	0.06	0.25	0.19
Vineland adaptive behavior composite, mean (SD), <i>n</i>	73.68 (8.01), 154	74.01 (9.27), 70	69.89 (7.42), 28	2.76	.07	−0.04	0.51	0.56
Vineland communication standard score, mean (SD), <i>n</i>	69.60 (11.45), 162	70.68 (13.42), 73	66.28 (9.79), 29	1.44	.24	−0.08	0.34	0.45
Vineland motor standard score, mean (SD), <i>n</i>	86.54 (10.43), 160	85.60 (11.19), 72	80.10 (10.39), 29	4.50 ^c	.01 ^c	0.08	0.62	0.53
Vineland socialization standard score, mean (SD), <i>n</i>	73.04 (6.85), 163	73.52 (8.11), 73	74.00 (7.57), 29	0.27	.76	−0.06	−0.13	−0.06

^a *P* values are for one-way analyses of variance comparisons when dependent variables were continuous; for qualitative variables, χ^2 tests were used.

^b Effect size indicators are in Cohen *d* metric (ie, SD units) with values of 0.2, 0.5, and 0.8 being indicative of small, medium, and large effects. χ^2 goodness of fit values were also transformed onto Cohen *d* metric. In the presence of 1 effect size, findings refer to the overall (omnibus) test statistic.

^c Signifies statistically significant *P* < .05.

complete testing were unlikely to be clinically important, as indicated with effect sizes being below an accepted medium-level threshold.³¹ Of the sample that did ultimately complete testing, there were no significant differences in medical characteristics or objectively measured cognitive, language, or motor functioning between subjects across result categories. We used billing and diagnostic codes to identify cases, and thus there is the potential for false-

negatives (children who have ASD were not billed in this manner), although this would be highly unlikely because toddlers are usually presenting for the first time, evaluated through the team assessment, and billed as such. The study sample comes from a single tertiary care site. However, the majority of children in our region with a concern for ASD are referred to a tertiary center for developmental evaluation because this is the means

to access services. Thus, we believe our sample is representative of young children receiving a diagnosis of ASD in our region. Although our sample size is large, the number of children with pathogenic genetic findings is small (*n* = 36) and must be considered when evaluating the frequency with which medical recommendations are made within this group. A strength of the study is the large sample size of toddlers with a DSM-5 diagnosis. Additionally, the

standardized format of the comprehensive interdisciplinary team visit allowed us to evaluate numerous medical and neuropsychological characteristics for the sample.

CONCLUSIONS

In a clinical sample of toddlers with DSM-5 ASD, almost 60% completed genetic testing (CMA and/or fragile X). Of those who completed genetic testing, 12% had a pathogenic finding. There was no association between medical characteristics or cognitive, language, and motor functioning and the likelihood of a pathogenic finding. Our results suggest that genetic testing (CMA and/or fragile X) should continue to be recommended for patients receiving a diagnosis of ASD, regardless of phenotypic characteristics at diagnosis. Further

medical recommendations were made in 72.2% of patients with pathogenic findings, indicating that genetic testing in this population has significant clinical implications. With these findings, we highlight the importance of genetic testing for toddlers with a diagnosis of ASD.

ACKNOWLEDGMENTS

We thank Collin Lee for assistance with data abstraction and Hyejin “Jasmine” Jeon for assistance with article preparation.

Study data were collected and managed by using REDCap electronic data capture tools hosted at BCH. REDCap is a secure, web-based application designed to support data capture for research studies, providing (1) an intuitive interface

for validated data entry, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data downloads to common statistical packages, and (4) procedures for importing data from external sources.

ABBREVIATIONS

ASD: autism spectrum disorder
Bayley: Bayley Scales of Infant and Toddler Development, Third Edition
BCH: Boston Children’s Hospital
CMA: chromosomal microarray
DSM-5: *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*
REDCap: Research Electronic Data Capture

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2020 by the American Academy of Pediatrics

FINANCIAL DISCLOSURES: Dr Harris is a coinvestigator on a study funded by Clinical Research Associates (CRA), LLC, an affiliate of the Simons Foundation. Dr Harris is also a coinvestigator on a study funded by Ionis Pharmaceuticals; the other authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Supported by the Palmer Family Fund for Autism Research. In light of the use of REDCap for this project, this publication was supported by National Institutes of Health/National Center for Research Resources Colorado Clinical Translational Science Institute Grant UL1 RR025780. Its contents are the authors’ sole responsibility and do not necessarily represent official National Institutes of Health views. Funded by the National Institutes of Health (NIH).

POTENTIAL CONFLICT OF INTEREST: Dr Harris is a coinvestigator on a study funded by Clinical Research Associates, LLC, an affiliate of the Simons Foundation. Dr Harris is also a coinvestigator on a study funded by Ionis Pharmaceuticals; the other authors have indicated they have no potential conflicts of interest to disclose.

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. Washington, DC: American Psychiatric Association; 2013
2. Risch N, Hoffmann TJ, Anderson M, Croen LA, Grether JK, Windham GC. Familial recurrence of autism spectrum disorder: evaluating genetic and environmental contributions. *Am J Psychiatry*. 2014;171(11):1206–1213
3. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The familial risk of autism. *JAMA*. 2014;311(17):1770–1777
4. Schaefer GB. Clinical genetic aspects of ASD spectrum disorders. *Int J Mol Sci*. 2016;17(2):180
5. Sztainberg Y, Zoghbi HY. Lessons learned from studying syndromic autism spectrum disorders. *Nat Neurosci*. 2016;19(11):1408–1417
6. Reddy KS. Cytogenetic abnormalities and fragile-X syndrome in autism spectrum disorder. *BMC Med Genet*. 2005;6:3
7. Johnson CP, Myers SM; American Academy of Pediatrics Council on Children With Disabilities. Identification and evaluation of children with autism spectrum disorders. *Pediatrics*. 2007;120(5):1183–1215
8. Yoo H. Genetics of autism spectrum disorder: current status and possible clinical applications. *Exp Neurol*. 2015;24(4):257–272
9. Schaefer GB, Mendelsohn NJ; Professional Practice and Guidelines Committee. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. *Genet Med*. 2013;15(5):399–407

10. Ho KS, Twede H, Vanzo R, et al. Clinical performance of an ultrahigh resolution chromosomal microarray optimized for neurodevelopmental disorders. *BioMed Res Int*. 2016;2016:3284534
11. Shen Y, Dies KA, Holm IA, et al; Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration. Clinical genetic testing for patients with autism spectrum disorders. *Pediatrics*. 2010; 125(4). Available at: www.pediatrics.org/cgi/content/full/125/4/e727-e735
12. Miles JH, Takahashi TN, Bagby S, et al. Essential versus complex autism: definition of fundamental prognostic subtypes. *Am J Med Genet A*. 2005; 135(2):171–180
13. Chodirker Bernard N, Chudley Albert E. Routine genetic testing for Asperger syndrome. *Genet Med*. 2008;10(11): 843–845
14. Tammimies K, Marshall CR, Walker S, et al. Molecular diagnostic yield of chromosomal microarray analysis and whole-exome sequencing in children with autism spectrum disorder. *JAMA*. 2015;314(9):895–903
15. Ho KS, Wassman ER, Baxter AL, et al. Chromosomal microarray analysis of consecutive individuals with autism spectrum disorders using an ultra-high resolution chromosomal microarray optimized for neurodevelopmental disorders. *Int J Mol Sci*. 2016;17(12): 1–14
16. Spence SJ, Trentacoste S, Rapin I. The genetics of autism. *Semin Pediatr Neurol*. 2004;11(3):196–204
17. McGrew SG, Peters BR, Crittendon JA, Veenstra-Vanderweele J. Diagnostic yield of chromosomal microarray analysis in an autism primary care practice: which guidelines to implement? *J Autism Dev Disord*. 2012; 42(8):1582–1591
18. Bayley N. *Bayley Scales of Infant and Toddler Development*, 3rd ed. San Antonio, TX: Harcourt Assessment; 2006
19. Sparrow SS, Cicchetti DV, Balla DA. *Vineland Adaptive Behavior Scales*, 3rd ed. San Antonio, TX: Pearson; 2016
20. Sparrow SS, Cicchetti DV, Balla DA. *Vineland Adaptive Behavior Scales, Second Edition (Vineland-II)*. San Antonio, TX: Pearson; 2005
21. Harstad EB, Fogler J, Sideridis G, Weas S, Mauras C, Barbaresi WJ. Comparing diagnostic outcomes of autism spectrum disorder using DSM-IV-TR and DSM-5 criteria. *J Autism Dev Disord*. 2015;45(5):1437–1450
22. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2): 377–381
23. Besterman AD, Wilke SA, Mulligan T-E, et al. Towards an understanding of neuropsychiatric manifestations in fragile X premutation carriers. *Future Neurol*. 2014;9(2):227–239
24. Baker EK, Arpone M, Aliaga SM, et al. Incomplete silencing of full mutation alleles in males with fragile X syndrome is associated with autistic features. *Mol Autism*. 2019;10(1):21
25. Field M, Dudding-Byth T, Arpone M, et al. Significantly elevated FMR1 mRNA and mosaicism for methylated premutation and full mutation alleles in two brothers with autism features referred for fragile X testing. *Int J Mol Sci*. 2019; 20(16):E3907
26. Cuccaro ML, Czape K, Alessandri M, et al. Genetic testing and corresponding services among individuals with autism spectrum disorder (ASD). *Am J Med Genet A*. 2014;164A(10):2592–2600
27. Narcisa V, Disenza M, Vaccari E, Rosen-Sheidley B, Hardan AY, Couchon E. Parental interest in a genetic risk assessment test for autism spectrum disorders. *Clin Pediatr (Phila)*. 2013; 52(2):139–146
28. Kalsner L, Twachtman-Bassett J, Tokarski K, et al. Genetic testing including targeted gene panel in a diverse clinical population of children with autism spectrum disorder: findings and implications. *Mol Genet Genomic Med*. 2018;6(2): 171–185
29. Jacquemont S, Coe BP, Hersch M, et al. A higher mutational burden in females supports a “female protective model” in neurodevelopmental disorders. *Am J Hum Genet*. 2014;94(3):415–425
30. Buja A, Volfovsky N, Krieger AM, et al. Damaging de novo mutations diminish motor skills in children on the autism spectrum. *Proc Natl Acad Sci USA*. 2018; 115(8):E1859–E1866
31. Cohen J. A power primer. *Psychol Bull*. 1992;112(1):155–159